Supplementary Methods:

Oligonucleotide probe retrieval assay:

Transfection: Control and WRN-depleted cells were transiently transfected (*via* precipitation of DNA with calcium phosphate) with primer-template DNA probes in which the template strand harbors a single *Taq*I (TCGA) restriction site, and the primer strand contains a 5' terminal biotin moiety. Both strands are protected from exonucleolytic digestion by the presence of dideoxycytidine at their 3' ends (Fig. 9). Twenty-four hrs post-transfection, DNA was extracted from total cell (cyto in Fig. 9) or nuclear lysates. Transfected DNA probes were isolated from total DNA by binding to streptavidin-coated magnetic beads (Invitrogen).

DNA probes/substrates:

a) Primer-template partial duplex DNA was generated by annealing a single-stranded oligonucleotide to M13mp18 ssDNA. As illustrated in Fig. 9, the oligonucleotide (43 nt) contains sequence that, in addition to being complementary to M13 DNA at its 3' end, has a unique barcoded sequence and a 5' terminal biotin cap. Linear M13mp18 ssDNA (949 nt) was obtained from circular phage DNA by annealing two synthetic oligonucleotides at base positions 2071-2110 and 3021-3060, and digesting the resultant double-stranded segments with the restriction endonucleases, *Bae*GI and *Afe*I, respectively. The 5'-biotinylated oligomer was hybridized to the linear ssDNA fragment (position 2670-2693) generated by restriction digestion.

b) The D-loop structure was formed by annealing a synthetic oligomer (43 nt) to a restriction fragment derived from digesting plasmid pCEP4 DNA (Invitrogen) with *Aat*II. The sequence at the 3' end of the oligomer is complementary to the insertion site of plasmid DNA (position 6272-6295). This oligomer also has a unique barcoded sequence and a biotin residue at the 5'-terminus (Fig. 9). The 5'-biotin tagged oligomer was mixed in 100-fold molar excess over plasmid DNA to increase the probability of

base pairing with plasmid DNA and facilitate displacement of the complementary plasmid DNA strand upon denaturation (Fig. 9).

qPCR: A sensitive quantitative PCR protocol, developed and implemented in our laboratory, was used to measure the mutation frequency at the *Taq*I restriction site (1,2). *In vivo* elongated DNA substrates were first extensively digested with *Taq*I restriction enzyme to eliminate DNA containing wild-type sequence. The DNA was then amplified using primers flanking the *Taq*I restriction site (Table 1). This generated a cycling threshold value for amplification of the test/target site [Ct (test)]. Similarly, using internal control primers that flank a region adjacent to the *Taq*I site (Table 1), we obtained control Ct values. The mutation frequency (MF) was calculated from the measured Ct values; MF (per base) $=1/(2^{\Delta Ct}x4)$, where ΔCt= Ct(control)-Ct(test).

References:

1. Bielas, J. H., and Loeb, L. A. (2005) *Nat Methods* **2**, 285-290
2. Wright, J. H., Modjeski, K. L., Bielas, J. H., Preston, B. D., Fausto, N., Loeb, L. A., and Campbell, J. S. (2011) *Nucleic Acids Res* **39**, e73

Supplementary Table 1: Primers Used in Oligonucleotide Retrieval Assay

Primer used to anneal to linear M13 ssDNA:
5'-biotin-GCACGTCAGGCACGGCGTCACATTCAACCGATTGAGGGAGGGA-3'
Oligonucleotides hybridized to M13 ssDNA for restriction cleavage:
Bae G1: 5'-TCAGTGCCTTGAGTAACAGTGCCCGTATAAACAGTTAATG-3'
Afe 1: 5'-AAGTCAGAGGGTAATTGAGCGCTAATATCAGAGAGATAAC-3'
qPCR Primers for M13 ssDNA elongation:
1. Test: 5'-GCACGTCAGGCACGGCGTC-3' (forward)
5'-AACGCGCTACAGTCTGACGCTAAA-3' (reverse)
2. Control: 5'-GCACGTCAGGCACGGCGTC-3' (forward)
5'-TCCGGCCTTGCTAATGGTAATGGT-3' (reverse)
Primer used to form D-loop DNA:
5'-biotin GCACGTCAGGCACGGCGTCTGAAGATCAGTTGGGTGCACGAGT-3'
qPCR Primers for D-loop elongation:
1. Test: 5'-GCACGTCAGGCACGGCGTC-3' (forward)
5'-AATAGTGTATGCGGCGACCGAGTT-3' (reverse)
2. Control: 5'-AACTCGGTCGCCGCATACACTATT-3' (forward)
5'-TGTCAGAAGTAAGTTGGCCGCAGT-3' (reverse)

Supplementary Figure Legends:

Supplementary Fig. 1: Synergistic degradation of bubble DNA requires the simultaneous presence of WRN and Pol δ. WRN or Pol δ (50-100 fmols of each) was incubated with bubble DNA at 37 ^{0}C for 20 min. Thereafter, the reaction mixtures were incubated at 63 ^{0}C for 10 min to render the enzymes inactive. Control reactions demonstrated that this temperature does not denature bubble DNA, but does abolish the enzymatic activities of WRN and Pol δ. Following inactivation, Pol δ or WRN was added to reactions that contained WRN or Pol δ, respectively. The reactions were incubated for an additional 20 min at 37 ^{0}C. As a control, WRN and Pol δ were incubated together with bubble DNA. W, WRN; δ, Pol δ.

Supplementary Fig. 2: Synergism with Pol δ requires more than just the exonuclease activity of WRN. Increasing amounts (0.3, 1.5, 6 pmols, indicated as 1, 2, and 3, respectively) of a minimal exonuclease domain of WRN (aa 1-240) were pre-incubated with Pol δ holoenzyme (60 fmol). The reactions were initiated by the addition of bubble DNA and incubated at 37 ^{0}C for 20 min as described in the legend to Fig. 1.

Supplementary Fig. 3: Co-operation of WRN with DNA Pol δ is not observed on all DNA substrates and with other 3'→5' exonucleases. WRN, Pol δ, or WRN + Pol δ (~6 fmol of each) were incubated with a D-loop mimic **(A)**, or Trex 1 (0.25 fmols) and/or WRN (15 fmols) were incubated with bubble DNA **(B)** at 37 ^{0}C and processed as described in Materials and Methods. Band intensities of hydrolytic products were quantified and plotted using ImageJ software.

Supplementary Figure 1
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Supplementary Figure 2
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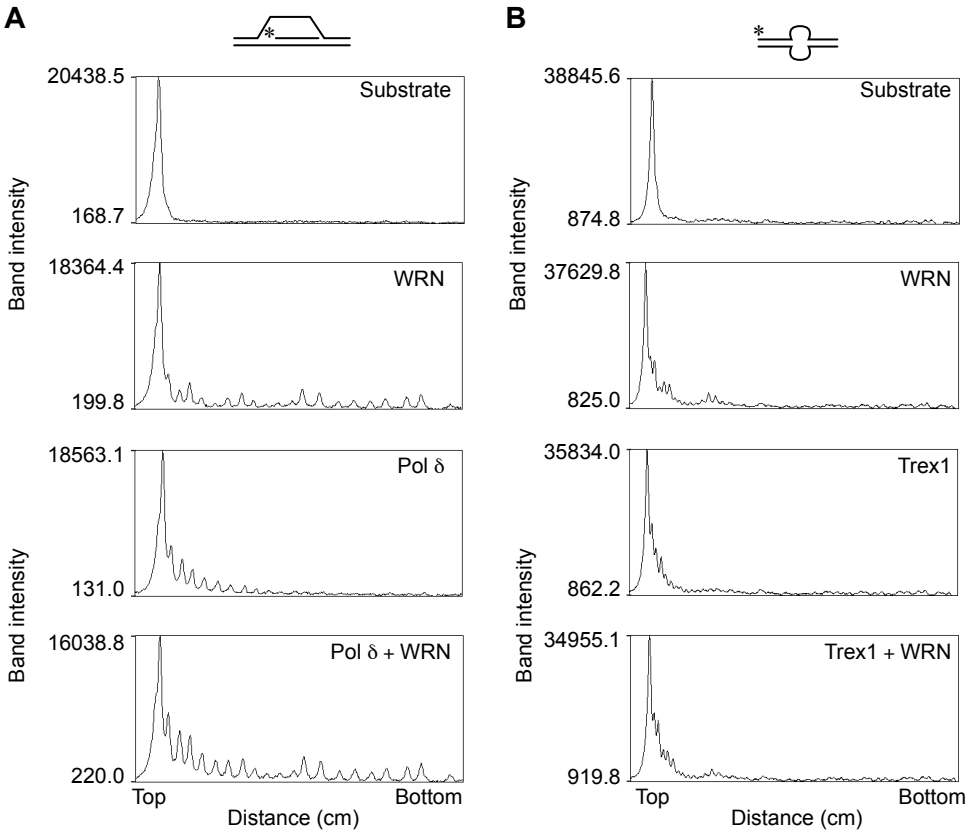

Supplementary Figure 3
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